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# Identification, Development, and Release of Insect Biocontrol Agents for the Management of *Phragmites australis*

Bernd Blossey

July 2014

Environmental Laboratory

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Final report



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# Abstract

Introduced *Phragmites australis* is rapidly spreading in North America, threatening wetland plant communities and endemic native genotypes (*Phragmites australis americanus*). Lack of successful long-term control resulted in initiation of biological control research. In the past, the program targeting introduced *Phragmites* has focused on several promising potential biological control agents with large impacts on *P. australis*. The purpose of this report is to: (1) identify potential agents for in-depth study; (2) outline and report initial testing procedures and results of host-specificity studies of identified agents; (3) assess possibilities to develop laboratory/greenhouse mass-rearing procedures; (4) outline approaches for long-term monitoring at pre-release sites; and (5) assess the extent of hybridization between native and introduced genotypes. All selected insect species are stem miners that overwinter as eggs, with larvae feeding in spring and early summer. Host specificity testing is being conducted in a Rhode Island quarantine facility and at the Center for Agricultural Bioscience International (CABI) in Switzerland. In addition, investigations continue on the impact of *Phragmites* populations on native fauna and flora as well as the economic and ecological effects of *Phragmites* invasion. Hybridization between native and introduced genotypes appears to be restricted to a single hybridization event in central New York State.

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# Preface

This technical report was funded by the U.S. Army Corps of Engineers, Aquatic Plant Control Research Program, Dr. Linda Nelson, Program Manager. This report was published by the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, Mississippi.

The work reported herein was performed by a number of investigators, with Bernd Blossey serving as the lead Principal Investigator, along with collaborators at Cornell University (Eric Nelson, Plant Pathology; and several graduate students), the University of Rhode Island (Richard Casagrande and Lisa Tewksbury), and overseas work at the Center for Agricultural Bioscience International (CABI) Switzerland (Hariet Hinz and Patrick Häfliger) and the Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Panamá (Kristin Saltonstall).

COL Jeffrey R. Eckstein was the Commander of ERDC, and Dr. Jeffery P. Holland was the Director.

# 1 Background

For decades, researchers have wrestled with questions regarding *Phragmites australis* (common reed) (Marks et al. 1994, Tewksbury et al. 2002). Is *P. australis* native to North America or was it introduced? Why has *P. australis* become so invasive and how do we measure its spread? The present-day existence of both native North American and introduced European haplotypes was confirmed through genetic methods (Saltonstall 2002). Initial introduction of European material occurred sometime in the early part of the 19th century, probably at Atlantic coast ports. All introduced populations examined in North America belong to the same haplotype (M), which is the most widespread haplotype worldwide. In the Northeast and Midatlantic regions of the United States, Type M has nearly entirely replaced native genotypes, while native genotypes appear more abundant in the Midwest and the Southwest (Saltonstall 2002). However, these populations may be declining (Marks et al. 1994), potentially accelerated by local introduction of nonindigenous genotypes (Marks et al. 1994, Blossey 2003a). The native genotypes have recently been recognized as a distinct subspecies *Phragmites australis americanus* Saltonstall, P.M. Peterson and Soreng (Saltonstall et al. 2004). Hybridization between native and introduced populations has been experimentally achieved in the lab (Meyerson et al. 2008) and there is now evidence for hybridization between North American and European genotypes in the field (Saltonstall et al. 2014).

The rapid expansion of introduced *P. australis* populations in both freshwater and brackish North American wetlands and the resulting ecological impacts are generally (but not always) considered detrimental (Marks et al. 1994, Chambers et al. 1999, Meyerson et al. 2000, Rooth and Stevenson 2000). Expanding populations threaten ecological, agricultural, recreational, and other ecosystem functions. Interest in controlling *P. australis* in urban, rural, agricultural, and natural areas in the Great Lakes region remains strong and is increasing in many other areas. The entire arsenal of control methods available to land managers (including herbicides, mowing, disking, dredging, flooding, draining, burning, covering, and grazing) has been tested in managing *P. australis* (Marks et al. 1994). Permanent control may be achieved in areas where tidal flushing with full-strength saltwater can be achieved, but this would be restricted to previously diked coastal marshes. Currently, the most widespread and

successful control method appears to be application of glyphosate (or another herbicide) late in the growing season, followed by prescribed burning or mechanical removal of dead stalks, and often subsequent application of herbicide the following year (Blossey and McCauley 2000, Ailstock et al. 2001). In order to maintain areas with low *P. australis* abundance, however, re-treatments are usually necessary every 3-5 years, representing a continued strain on management budgets. In addition, negative side effects on non-target plants are inevitable if non-selective herbicides are used over large areas. The inability to achieve long-term control of invasive *P. australis* resulted in the initiation of a biocontrol program. Since 1998, this program has researched the possibilities of using natural enemies from the native range as control agents.

In the past 3 years, the program targeting introduced *Phragmites* has focused on several promising potential biological control agents identified in Europe as having significant impacts on *P. australi*s growth and performance. Host specificity testing was conducted in a Rhode Island quarantine facility (Richard Casagrande, PI and Lisa Tewksbury), while additional host specificity work and maintenance of a rearing colony of the appropriate insects was maintained in Europe at the Center for Agricultural Bioscience International (CABI) in Switzerland (Patrick Häfliger and Hariet Hinz) and studies assessing hybridization were conducted at the Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Panamá (Kristin Saltonstall). In addition to this work, surveys were conducted for potential soil pathogens influencing *Phragmites* success (Eric Nelson and Ellen Crocker). Investigations also continued on the impact of various *Phragmites* populations on native fauna and flora. An economic and ecological assessment of *Phragmites* invasion and management was also conducted (Bernd Blossey, Jeremy Dietrich, Laura Martin and Jillian Cohen). Experiments are continuing and much of the resulting information will be summarized and published in the near future. The purpose of the current effort (and a supplement) was to enhance the biocontrol program with the following five major objectives:

- **Objective 1:** Identify potential agents for more in-depth study.
- **Objective 2:** Develop testing procedures and conditions for host-specificity studies and collect data on host specificity of identified agents.
- **Objective 3:** Develop laboratory/greenhouse mass-rearing procedures.

- **Objective 4:** Assist in selection of pre-release sites for long-term monitoring.
- **Objective 5**: Assess the extent of hybridization between native and introduced genotypes.

# 2 Objective 1: Identify Potential Agents for More In-depth Study

## Accomplishments during the reporting period

At the time of report writing, the field season had just begun; thus, much of the work program had only been initiated and results will not become available until a later date. Ongoing work will be described in as much detail as possible, but even for results already obtained, much more sophisticated analyses will be reported on in the future.

In Europe, where the invasive haplotype M most likely originated (Saltonstall 2002), *P. australis* is attacked by more than 150 different herbivore species (Tewksbury et al. 2002). Based on their feeding niche, damage inflicted, and reported host-specificity, nine insect species were initially selected as potential biological control agents (Schwarzlaender and Häfliger 2000, Tewksbury et al. 2002). This list has now been further reduced to the four most promising species based on the impact of these agents and some preliminary host-specificity testing. Additional criteria used in selecting insect species are knowledge of initial specificity, impact on *Phragmites* growth and performance, feeding niche, and potential competitive interactions with other agents being considered. The following is a summary of life history and ecology (where known) of the most promising potential natural enemies associated with *P. australis* in Europe. Species that have been introduced to North America accidentally have been excluded due to their lack of impact on plant performance (for a listing of these species, see Tewksbury et al. 2002). However, these species will play an important role in evaluating biocontrol agents that have been proposed for further evaluation, since they may interact in various ways. Potential negative or competitive effects that could reduce the success of control agents need to be avoided.

## Potential *P. australis* biocontrol agents identified in Europe

The work described here focuses on four different, but closely related, noctuid shoot-boring moths (*Archanara geminipuncta, Arch. dissoluta, Arch. neurica, and Arenostola phragmitidis*). These moths have the highest impact and probability for success according to evaluations conducted over the past 8 years (Häfliger et al. 2005, 2006a, 2006b). All

species develop in the stems, where larvae feed over the summer months. *Archanara geminipuncta* is a widespread and well-known *P. australis* herbivore in Europe, but little was known about biology and impact of the other *Archanara* species or *Aren. phragmitidis* when our investigations began. The whitish, flesh-colored larvae of the *Archanara* species were difficult to distinguish. Based on adult records, *Arch. neurica* and *Arch. dissoluta*, while widespread in Europe, appeared less abundant than *Arch. geminipuncta* (Grabo 1991).

***Arenostola phragmitidis*** appears to have a more northern and eastern distribution in Europe compared to the *Archanara* species (Bretherton et al. 1983). Although *Aren. phragmitidis* is locally common in England and widespread in marshes in the Netherlands and in Denmark, little is known about abundance, impact, larval development, and mortality factors. Eggs of *Aren. phragmitidis* are the first to hatch after overwintering and early instars often feed gregariously, mostly above the growing point, in the first shoot until they reach the second instar. Larvae pass through four instars and need to feed on three to four shoots to complete development. In contrast to the *Archanara* species, pupation occurs in soil or litter, and rearing larvae build a thin silken cocoon mixed with soil particles. The species is univoltine and adults fly in July and August (Bretherton et al. 1983).

***Archanara neurica,*** the least common of the three known *Archanara* species feeding on *P. australis* in Europe, is also univoltine with adults flying in July. Larvae of *Arch. neurica* hatch nearly simultaneously with *Aren. phragmitidis* larvae, but they feed individually from first to third instar in their first *P. australis* shoot. Larvae pass through four instars and change shoots once or twice during their development with each additional shoot providing food for 1-2 weeks. Creation of *Archanara* pupal chambers alone does not interfere with stem growth and larvae do not feed in this period, which usually lasts 2-5 days. Pupation of *Arch. neurica* occurs head downwards in lower portions of either attacked or unattacked stems.

***Archanara dissoluta*** is also univoltine and is the second most commonly mentioned *Archanara* species in Europe. Adults fly in July and August and lay eggs in two to three rows under leaf sheaths (Michel and Tscharntke 1993). Larvae emerge approximately 2 weeks after *Arch. neurica* and *Aren. phragmitidis* larvae and feed individually for 4-5 weeks

in their first shoot until they reach the fourth instar. Larvae pass through five instars and change shoots once or twice before completing development. Pupation occurs mostly head downwards in a *P. australis* internode at ground level, a distinct difference from the other two *Archanara* species. Mature larvae may pupate in green shoots, but they may also select shoots from previous growing seasons.

***Archanara geminipuncta*** is univoltine, with adults flying in July and August. Soon after emergence, females mate and begin to lay eggs in individual rows under leaf sheaths (Skuhravy 1981, Bretherton et al. 1983, Ostendorp 1993). Eggs overwinter under leaf sheaths and larvae hatch nearly simultaneously with *Arch. dissoluta* larvae in spring. First instars can float on the water surface and are able to climb onto shoots. First instars start feeding in the soft, nutrient-rich tissues above the growing point in April soon after shoots begin to grow and the facultative gregarious larvae develop for 2-3 weeks in their first *P. australis* shoot until they reach the third instar. As larvae grow, they quickly exhaust food resources of individual shoots and larvae change shoots three to four times during their development (Michel and Tscharntke 1993). Mature larvae locate an undamaged internode and prepare an oval emergence window in the lower portions of internodes, but leave the epidermis intact (Tscharntke 1990). Pupation occurs head up and the emerging adult pushes its way out onto the stem where it hardens and wings unfold.

Attacked stems (not those where only pupation occurs) show characteristic signs of damage, which differ depending on larval development stage. Stems attacked by early instars wilt and die completely; stems attacked by later instars wilt, loose stem tips, and might develop one to four side shoots. While reports on the impact of *Arch. geminipuncta* on *P. australis* performance differ, up to 90% of stems can be attacked with shoot heights and aboveground biomass reduced by 50-60% and 20-60%, respectively (Tscharntke 1990, 1999). Large population fluctuations with outbreak cycles of 3-4 years have been reported (Michel and Tscharntke 1993).

Investigations into the life history and biology of the four noctuid species under climate conditions in Switzerland are summarized as follows:

- Adult emergence for each species was highly synchronized during a period of 2-3 weeks (but varied from year to year). There was only a short period where moths of all species were active simultaneously.

- *Arch. neurica* and *Aren. phragmitidis* were always the first two species to appear and displayed nearly identical emergence phenology, followed by *Arch. geminipuncta* and *Arch. dissoluta*, which emerged approximately 2 weeks after the earlier emerging species.
- Adults were short-lived, on average 10-14 days, and observations suggest that they do not need to feed during their brief adult life.

Females began oviposition within 24 hr after emergence and oviposition was concentrated during the first 3-4 days after mating. This was especially true for *Arch. geminipuncta,* where the majority of eggs were laid during the first 2 days after mating. The most balanced oviposition pattern of all four noctuids was displayed by *Arch. dissoluta,* with a similar number of eggs per female for 1 week and oviposition of most species complete after 10 days. All *Archanara* females typically lay eggs on already dry leaf sheaths (but current growing seasons's stems) in the lower parts of the stems, while *Aren. phragmitidis* females prefer green leaf sheaths higher on the stems. For both *Arch. neurica* and *Arch. geminipuncta*, which lay eggs in single rows, egg cluster size is significantly smaller than egg cluster size for *Arch. dissoluta* and *Aren. phragmitidis,* which usually oviposit in two rows covered by a secretion. These differences in clutch size and additional differences in egg coloration allow separation of species in the egg stage. The total average number of eggs laid per female ranges from as low as 100 for *Arch. dissoluta* to more than 160 for *Aren. phragmitidis*.

Although adult flight periods in mid-summer were different among the four noctuid species, egg hatch after overwintering was highly synchronized and coincided with *P. australis* shoot growth in spring. *Arenostola phragmitidis* was always the first species to emerge, with the *Archanara* species following within a few days. Egg hatch was always complete by early May. A rapid succession occurred from first to fourth instar, with each phase lasting about 1 week. Instar duration increases with successive molts, with approximately 2 weeks needed to complete the fourth instar and approximately 3 weeks needed to complete the fifth instar. Pre-pupal and pupal periods combined last about 5 weeks before adults begin to emerge. There is little overlap except for successive developmental stages.

In the European surveys, which covered a large area from the Danish border to Italy and from the Atlantic to Hungary, stems infested by

noctuid stem borers were found at all sites except for one. With the exception of three sites around Lake Neuchâtel in Switzerland, where *Arch. dissoluta* dominated, *Arch. geminipuncta* was the most common noctuid occurring at 66.6% of all field sites investigated. The other species were less common, with *Arch. dissoluta* occurring at 40.0%, *Arch. neurica* occurring at 33.3%, and *Aren. phragmitidis* occurring at 26.7% of sites. Sites in Switzerland and Northern Germany in general showed higher noctuid attack rates (maximal 40-50%) than sites in Austria and Hungary (maximal 7%). Mean noctuid attack rate was independent of the *P. australis* stand size and stem density. Most importantly, overall attack rates increased with an increase in the number of noctuid species encountered in samples, suggesting lack of competitive exclusion. These increases also indicate that introduction of multiple species that exploit a similar feeding niche may improve overall control of *P. australis* in North America.

Bird predation, parasitism, and mortality due to unknown causes were the main mortality factors of noctuid larvae and pupae. Early instars suffered substantially less mortality than later instars and pupae. The main mortality factors were an ectoparasitic ichneumonid attacking third and fourth instar larvae mainly in late May. Mature larvae and pupae suffered a high mortality rate (>50%) particularly through bird predation, with additional losses due to attack by a tachinid fly and a pupal parasitoid.

Future investigations will assess the danger that larvae and pupae may encounter after a potential release in North America. Related *Archanara* species are present in North America and mortality factors for these species will be assessed if funding to conduct such work is available. In contrast to the European situation where reed beds are an important migratory habitat for many birds, the same habitats are nearly devoid of specialized bird species in North America, particularly of species able to predate on noctuid pupae in *P. australis* stems. This fact would suggest that a major mortality factor limiting the impact of the noctuids on *P. australis* performance in Europe may not be present in North America. However, various North American bird species are increasingly being encountered. This is particularly true for black-capped chickadees (*Parus atricapillus*), downy woodpeckers (*Picoides pubescens*), and hairy woodpeckers (*Picoides villosus*) in introduced *P. australis* stands along the East Coast. These birds feed on introduced insects overwintering in *P. australis* stems (*Lasioptera hungarica* and *Lipara* spp.). Due to their

feeding mode and ability to open stems, they may represent predator species able to take advantage of stem-feeding Lepidoptera if they forage in reed beds during the growing season.

Investigations of four sympatric noctuid species attacking *P. australis* stems in Europe revealed subtle differences in their life history and phenology. In particular, adult emergence, egg hatch after overwintering, larval feeding habits (facultatively gregarious or solitary in early instars), and the timing and number of shoot changes distinguish the species. The most substantial difference in life history and ecology is probably the restriction of *Aren. phragmitidis* to dry sites due to pupation requirements. However, these noticeable differences do not result in differences in the impact of individual larvae on *P. australis* growth, since larval feeding of all species severs stem tissue and results in premature tip death and lack of reproduction in attacked stems. Despite substantial overlap in phenology and biology, there were large differences in field attack rates and presence/absence of individual species at the field sites investigated. While *Arch. geminipuncta* has been confirmed as the most widespread and abundant *Archanara* species attacking *P. australis*, the two least common species (*Arch. neurica* and *Aren. Phragmitidis)* still occurred at 25-30% of the field sites, albeit at much lower abundance than the more widespread *Arch. geminipuncta*.

At present, differences in abundance between the widespread and the less common species cannot be explained, although our data indicate that the later hatching and emerging species *Arch. geminipuncta* and *Arch. dissoluta* reach the highest attack rates and are most widely distributed. There were no obvious geographic or climatic factors associated with the presence/absence or abundance of individual species. Based on laboratory rearings, one would expect that the species should show similar attack rates in the field. Nearly the same natural enemies were encountered for all species, although there is no information on pupal mortality of *Aren. phragmitidis*. Pupation in the soil, while requiring dry sites, is expected to effectively reduce bird predation, but exposes larvae and pupae to ground-dwelling vertebrate and invertebrate predators. High predation and parasitoid attack have been reported, although their role in top-down control for *Arch. geminipuncta* populations remains unclear (Galichet and Radisson 1976, Tscharntke 1990). The above- reviewed four noctuid species, in particular *Arch. geminipuncta*, are considered to be the highest priority candidates for a biocontrol program targeting invasive *P. australis* in North America.

During work in Europe, a number of additional potential control agents were evaluated, in particular ***Platycephala planifrons*** (Fabricius) (Diptera:Chloropidae). Larvae of this fly attack *Phragmites* shoots early in the year, leading to severe stunting of attacked stems by killing the growing point. Females fly in the summer and are long-lived. Eggs are laid in late summer. Larvae hatch in late summer, feed for a limited period, and then overwinter. *Platycephala planifrons* was one of the most damaging species found during surveys in Europe. Attack can cause biomass reductions of >50%, but overall attack rates at a field site remain low. This is potentially associated with requirements of first instar larvae to overwinter in dormant shoots below ground. The species was evaluated for its preference among different *Phragmites* genotypes, but due to its low attack rates, it is currently considered a "second tier" candidate (Häfliger et al. 2005).

The three species discussed below were also evaluated. However, due to difficulties in working with them or lack of availability, they are not currently considered to be viable candidates for a biocontrol program. This assessment may be reevaluated if the higher priority species fail to meet stringent safety requirements. ***Phragmataecia castaneae*** (Hübner) (Lepidoptera, Cossidae) is a large moth needing 2 years to complete its development, which occurs at the base of the shoot and in the rhizomes. Moths fly in summer and females lay 200-400 eggs. Larvae may move from shoot to shoot as they look for new food during their development. Larvae can be found in both dry reed stands and those that are permanently flooded. ***Chilo phragmitella*** (Hübner) (Lepidoptera: Pyralidae) mines shoots and roots of *P. australis* in Europe. Larvae are active in the summer; older larvae mine deeper parts of the rhizome and are difficult to detect. Infested shoots remain small and wilt. Larvae of ***Schoenobius gigantella*** (Denis & Schiffermüller) (Lepidoptera: Pyralidae) mine shoots of flooded *Phragmites* below the water level, causing considerable damage. Attacked shoots wilt and break apart. Little is known about the life history of the species, but it is assumed that larvae need 2 years to complete development. Adults fly in the summer.

Of the other herbivores (including pathogens) encountered during field surveys in Europe or mentioned in the literature, none appears to make a more promising candidate than the four shoot-mining moths that will be targeted initially. European surveys did not encounter pathogens that appear to be potent natural enemies of *P. australis*, but one cannot assume that they do not exist (survey personnel focused on insect herbivores and

had expertise in entomology). Surveys of soil pathogens, particularly oomycetes in North America (focusing on the Finger Lakes region in New York; Nelson and Crocker, unpublished results) discovered hundreds of species including *Pythium phragmitidis*, a species suspected of contributing to *P. australis* declines in Europe (Nechwatal and Mendgen 2009). Nelson and Crocker are currently testing the pathogenicity of different isolates on *P. australis* germination and results are extremely diverse. Isolates and *P. australis* population appear to be significant factors, while origin (native or introduced for the plant seed source) may not have a significant role in explaining pathogenicity. While these *Pythium* species have large effects on seed germination, it is doubtful that they can contribute to reductions in *P. australis* vigor for well-established populations. Researchers are also assessing the impact that these species may have on native wetland plants to assess the potential for pathogen interference with wetland restoration.

## Re-survey of *Phragmites* herbivores in populations along the East Coast

It was proposed that survey areas in eastern North America that were sampled almost a decade ago be re-surveyed to assess any potential changes in the Phragmites herbivore communities in North America. This re-survey will evaluate whether additional European or Asian herbivores were accidentally introduced or have spread in the past decade. The potential of these herbivores for use in controlling introduced *Phragmites* or their threat to native *P. australis americanus* will also be examined.

From October 2010 to April 2011, all stems from five 1-$m^2$ quadrats (transect across a *P. australis* stand) were collected at nine locations in Massachusetts, Rhode Island, Connecticut, and New Jersey. It appears that new herbivores can now be located, although the emergence of several species that were found overwintering as larvae/pupae in the stems is still being awaited. These adults will either be identified by the authors or sent to specialists for identification. The most common species encountered are the mealybug *Chaetococcus phragmitidis*, the stem tip galling flies *Lipara rufitarsis* and *L. similis*, the stem-mining midges *Lasioptera hungarica* and *Microlasioptera flexuosa*, and the rice grain gall midge *Giraudiella inclusa*. Dissections have only recently been completed and the emergence of adults for unknown species is still ongoing; therefore, more detailed results on the differences among sites and attack rates will be summarized in a forth-coming report.

# 3 Objective 2: Develop Testing Procedures and Conditions for Host-Specificity Studies and Collect Data on Host Specificity of Identified Agents

## Unique challenges in developing biological control of invasive *P. australis*

Implementing a biological control program that targets invasive *P. australis* in North America will encounter a set of unique challenges. Native endemic North American genotypes of *P. australis* (Saltonstall 2002, 2003; Blossey 2003a, 2003b), which were elevated to subspecies status as *Phragmites australis americanus*, will need to be protected. This requires all potential biocontrol agents to be specific at the subspecies level. The distinct differences in chemistry, morphology, and herbivore communities suggest that differences exist between genotypes, and that consumers respond to these differences (Blossey 2003a, 2003b). Several specialized *P. australis* herbivores appear genotype specific. Among these are the gall midge *Lasioptera hungarica*, a species introduced from Europe and exclusively associated with invasive *P. australis* clones (Blossey 2003a). At least two native North American "signature" species, a gall midge, *Calamomyia phragmites,* and a long-legged fly, *Thrypticus* sp., are exclusively found in native genotypes. These preferences (or differences in herbivore resistance among clones) are maintained even when clones grow within close proximity (a few meters) of each other (Blossey 2003a). These data from North America provide some evidence that genotype-specific biological control of *P. australis* may be possible. However, this discrimination appears to break down when native and introduced genotypes hybridize. Observations in New York suggest that *L. hungarica* will attack hybrid stems (Saltonstall et al. 2004). It is too early to assess the full scale of implications if hybridization is more widespread than currently recognized (see Objective 5), but the continuing cryptic invasion of European genotypes has already resulted in the disappearance of endemic genotypes in New England and the survival of native genotypes, at least in the East, appears threatened (Saltonstall 2002).

*Phragmites australis* is the only species in its genus in North America. This taxonomic isolation further reduces the potential for non-target effects (Pemberton 2000) and should facilitate selection of herbivores with adequately limited host range. However, the presence of the native subspecies *P. australis americanus* presents some important challenges (addressed above) and a major emphasis in the proposed work focuses on the safety of the native subspecies. *Phragmites australis* is a member of the Poaceae family, with more than 100 genera represented in the northeastern United States alone (Gleason and Cronquist 1991). The species most closely related to *P. australis* is *Arundo donax* L.**,** an invasive introduced species. Following TAG guidelines, a tentative list of approximately 45 plants was developed and reviewed for host range testing; this list has been revised based on comments that were received. The list has a primary emphasis on native wetland species and commercial crops, and a secondary emphasis on ornamental grasses. The most important genera to consider for wildlife value include species of *Typha*, *Spartina*, *Carex*, *Scirpus*, *Eleocharis*, *Juncus*, *Zizania*, *Arundinaria*, and *Calamagrostis*.

Experimental procedures were based on plant collections in Europe (CABI Bioscience Centre in Delémont, Switzerland) and at the University of Rhode Island (URI) in Kingston, Rhode Island. Tests in Europe will allow study of the behavior of control agents in common gardens, in field cages, or under unconfined conditions. Many of these tests are either in preparation or ongoing. More results were anticipated by the fall of 2013, including larval feeding (to be completed in July) and adult oviposition (adult flight periods in August). Work at URI, where an approved quarantine facility is available, is focusing on testing native North American species.

Plants for host range testing were either field collected or started in the URI greenhouse and then initially transplanted into containers held outside the greenhouses or, in the case of field-collected material, transplanted directly into containers and held outdoors. Plant quality is critically important in host acceptance studies (Blossey et al. 1994a, 1994b). Growing plants outdoors avoids the complex of common greenhouse pests (aphids, thrips, spider mites, etc.) and also allows plants to better develop their normal growth and chemical characteristics (Blossey et al. 1994a, 1994b). Growing plants in containers gives the flexibility to move plants into quarantine when needed for experiments. Concurrent with the development of the TAG list, obvious candidate hosts were planted, including various *Spartina spp*.,

*Typha*, *Scirpus*, *Zizania*, cereal crops, etc. However, problems were encountered in achieving large enough stem diameters for many plant species when they were grown in pots/containers. The stem-mining moth larvae are extremely "choosy" when it comes to stem diameters, including for their original host plant *P. australis*. To allow for reliable results, efforts were shifted to growing plants in a common garden in "trenches" or "ditches" at the Agronomy farm at URI. This change achieved large stem diameters, which the larvae (at least for their host plant) now accept for oviposition (Figure 1). These 35 trenches are 1 m by 1 m by 0.8 m deep and utilize a rubber pond liner to provide the optimum environment for wetland plants. Those plants that are normally flooded in their natural habitats are provided with a flooded pond, and those that are not normally flooded are grown in ponds that are filled with wet soil. A number of non-aquatic plant species are also grown in the same area without pond liners. Almost 30 species of perennials are now established in the common garden (Table 1). Many of the remaining species will be started from seed, and quite of few of these are crop species (e.g. corn or barley) and thus easily obtainable. Moreover, Patrick Häfliger at CABI has already tested *A. geminipuncta* in no-choice conditions on eight largely agricultural species. A full list of all host specificity results will be available in the Annual Report, which will focus on the most recent work completed at URI to provide an update on achievements resulting from the availability of additional funding.

Figure 1. Common garden at URI.

Table 1. Plant species available in a common garden in Kingston, RI for host specificity testing in quarantine.

| Plant Species | Common Name |
|---|---|
| 1. *Agropyron cristatum* | Crested wheatgrass |
| 2. *Andropogon gerardii* | Big bluestem |
| 3. *Arundinaria gigantea* subsp. *tecta* | Switch cane |
| 4. *Carex lurida* | Shallow sedge |
| 5. *Cortaderia selloana* | Pampas grass |
| 6. *Dactylis glomerata* | Orchardgrass |
| 7. *Distichlis spicata* | Saltgrass |
| 8. *Elymus virginicus* | Virginia wildrye |
| 9. *Eragrostis trichodes* | Sand love grass |

| Plant Species | Common Name |
|---|---|
| 10. *Glyceria striata* | Fowl mannagrass |
| 11. *Iris versicolor* | Blueflag iris |
| 12. *Juncus effusus* | Common rush |
| 13. *Leersia oryzoides* | Rice cutgrass |
| 14. *Lolium perenne* | Perennial ryegrass |
| 15. *Panicum virgatum* | Switchgrass |
| 16. *Phalaris arundinacea* | Reed canarygrass |
| 17. *Phragmites australis* | Common reed |
| 18. *Pontederia cordata* | Pickerelweed |
| 19. *Schoenoplectus acutus* | Hardstem bulrush |
| 20. *Schoenoplectus americanus* | Chairmaker's bulrush |
| 21. *Sorghastrum nutans* | Indiangrass |
| 22. *Sparganium americanum* | American burreed |
| 23. *Spartina alterniflora* | Smooth cordgrass |
| 24. *Spartina cynosuroides* | Big cordgrass |
| 25. *Tripsacum dactyloides* | Eastern gamagrass |
| 26. *Typha angustifolia* | Narrowleaf cattail |
| 27. *Typha latifoliia* | Broadleaf cattail |

The insect herbivores that are presently being evaluated for Phragmites biocontrol are all univoltine (one generation per year) and all overwinter as eggs on dead stems and leaves. These insects are not being reared in quarantine, but rely on annual shipments of eggs of these shoot-boring moths from Patrick Häfliger of CABI–Europe in Switzerland for host range testing. These eggs are held outdoors in Switzerland before shipment to URI in late winter. Typically eggs are received in March of each year (usually 500-1000, depending on rearing success of *Arch. geminipuncta*). Due to rearing difficulties, some of the previous additional colonies of other control agents collapsed last year and these rearing colonies will need to be replenished to allow for more and extended host range testing. Patrick Häfliger is working on new collections and an increase in his rearing operation at CABI.

Once eggs are received, they are placed into a 4 °C chamber and removed as needed to allow the hatch of the first instars for host specificity testing. Test plants with new shoot growth are dug from the common garden (Figure 1) and transplanted into nursery containers. Timing for this

process is extremely important, as larvae only accept shoots of the appropriate diameter and development stage. While this behavior increases the overall safety of these insects, it complicates work in quarantine and for host specificity testing overall, as plant growth and egg hatch need to be synchronized. With some experience and a steep learning curve, success has constantly improved. Without the ability to store eggs in a refrigerator, this would be entirely impossible.

Host range testing is conducted in two stages. In the first stage, individual newly hatched larvae are confined in small containers with appropriately-sized stems to see if they can bore into the stems and survive for 5 days (Figure 2). In stage 2, those plants that display positive results (i.e. larvae attack and enter stems) in Stage 1 are tested to see if larvae can complete development on them.

Figure 2. Testing chamber

Stem height and basal stem diameter are measured before setting up each test. One replicate consists of one larva exposed to one to three stems enclosed inside a 5-cm-diam acrylic tube that is either 30.5 cm or 46 cm tall (Figure 2). The tube is buried into the soil, supported by bamboo sticks, and ventilated by fabric screening under a cap at the top of the tube. A first instar of *A. geminipuncta* is placed at the base of young plant shoots using a wet fine-tip paint brush. Larvae are given 5 days to enter stems and feed before each replicate is evaluated. All stems are dissected, and stem attacks (represented by feeding damage, frass, or entrance and exit holes) are recorded and photographed.

In initial no-choice larval feeding trials conducted in 2011, 15 nontarget species and the exotic *Phragmites* controls were tested. Fifteen replicates of each species were tested, as well as 45 replicates of exotic *Phragmites* control for *Arch. geminipuncta* (Table 2). Success of the testing method was improved with the introduced Phragmites controls: 93% feeding of replicates with *A. geminipuncta*, and 78% of larvae still alive at the end of the 5-day test period. Three nontarget species received some feeding damage by *Arch. geminipuncta* (Table 2); several of these are now undergoing larval development tests (Stage 2 testing). No attempts were made to rear *A. geminipuncta* on wheat (*Triticum aestivum*), as this species is clearly outside the potential host range. Not only are fields

usually harvested at the time of insect flight (August); there is no possibility for eggs to survive the winter even if plants are harvested later. Thus, insects will not be able to complete their development in annual wheat. Furthermore, wheat is obviously an important crop all through the native range of *A. geminipuncta* and the species was never reported to attack wheat. Wheat and *P. australis* can be found in close association; thus, there would be plenty of opportunity for the larvae to attack wheat in Europe or Asia. There are no reports of such events; therefore, *T. aestivum* is outside the possible host range of *A. geminipuncta*.

Table 2. Host specificity test results with *Archanara geminipuncta* using one larva/replicate; N = # replicate (tube); Feeding damage indicated only when feeding occurred inside the stem.

| Species Tested | N | No. Stems | Stem Height (avg. in cm) | Stem Base Diameter (avg. in mm) | No. Reps with Feeding | No. Live Larvae |
|---|---|---|---|---|---|---|
| *Phragmites australis (exotic)* | 45 | 70 | 12.10 | 3.4 | 42 | 35 |
| *Agropyron cristatum* | 15 | 30 | 7.96 | 1.1 | 0 | 0 |
| *Andropogon gerardii* | 15 | 17 | 8.26 | 2.0 | 0 | 0 |
| *Arundinaria gigantea subsp. tecta* | 15 | 20 | 18.91 | 2.2 | 1 | 0 |
| *Avena sativa* | 15 | 28 | 12.60 | 3.3 | 0 | 0 |
| *Danthonia spicata* | 15 | 25 | 8.55 | 0.9 | 0 | 0 |
| *Eragrostis trichodes* | 15 | 28 | 8.56 | 2.0 | 0 | 0 |
| *Hordeum vulgare* | 15 | 38 | 11.35 | 2.3 | 0 | 0 |
| *Lolium perenne* | 15 | 33 | 5.58 | 2.1 | 0 | 0 |
| *Oryza sativa* | 15 | 31 | 18.04 | 4.6 | 0 | 0 |
| *Schoenoplectus americanus* | 15 | 42 | 26.75 | 3.1 | 2 | 0 |
| *Secale cereale* | 14 | 27 | 4.68 | 1.4 | 0 | 0 |
| *Setaria italica* | 15 | 16 | 8.26 | 3.6 | 0 | 0 |
| *Triticum aestivum* | 15 | 62 | 10.55 | 2.5 | 4 | 1 |
| *Zea mays* | 15 | 15 | 15.47 | 8.0 | 0 | 0 |
| *Zizaniopsis milacea* | 15 | 35 | 3.83 | 4.67 | 0 | 0 |

Three replicates were run with *A. geminipuncta* for each plant species, and six replicates of exotic *Phragmites* were run as control. Five neonate insects were placed at the base of a stem in a flat, with multiple stems of the test or control plant (Figure 3). These flats were placed in an aluminum cage. Some inconsistencies were noted regarding the quality of the stems in each flat in this experiment. The experiment is ongoing.

Figure 3. Stage 2 testing flat with *Phragmites australis* at URI quarantine.

Table 3. Summary of host range tests for *Archanara geninipuncta*: Yes or No for stem feeding and percent of replicates with stem feeding.

| Species Tested | *A. geminipuncta* | |
|---|---|---|
| | Feeding | % |
| *Phragmites australis (exotic)* | Yes | 93 |
| *Agropyron cristatum* | No | |
| *Andropogon gerardii* | Yes | 7 |
| *Arundinaria gigantean subsp. tecta* | No | |
| *Avena sativa* | No | |
| *Danthonia spicata* | No | |
| *Eragrostis trichodes* | No | |
| *Hordeum vulgare* | No | |
| *Lolium perenne* | No | |
| *Oryza sativa* | No | |
| *Schoenoplectus americanus* | Yes | 13 |
| *Secale cereale* | No | |
| *Setaria italica* | No | |
| *Triticum aestivum* | Yes | 27 |
| *Zea mays* | No | |
| *Zizaniopsis milacea* | No | |

Table 4. Plant species in 2011 Stage 2 testing.

| Species Tested |
|---|
| *Phragmites australis (exotic)* |
| *Phragmites australis (native) NYE* |
| *Phragmites australis (native) NBS* |
| *Phragmites australis (native) ME* |
| *Arundo donax* |
| *Cortaderia selloana* |
| *Iris versicolor* |
| *Phalaris arundinacea* |
| *Schoenoplectus acutus* |
| *Spartina alterniflora* |
| *Spartina cynosuroides* |

## Assessment of host specificity of accidentally introduced herbivores and resistance of native and introduced *P. australis* to these herbivores

Additional funding that became available was used to create a common garden of native and introduced *P. australis* populations from across North America. Plants in the common garden were used to assess herbivore attack rates of native and introduced populations growing under identical conditions. The common garden (Figure 4) consists of 28 *P. australis* populations (14 native populations, 14 introduced populations) from across North America (Table 5). Five replicate clones of each population were grown in separate trenches and paired with a geographically paired population of different origin. After initial planting, plants were allowed to expand and the results of clonal expansion and competition (still in progress as of fall 2013) were analyzed. Local herbivores (aphids and *Lipara* spp.) colonized this garden, which allowed for assessment of their distribution across the various genotypes when grown under identical conditions.

This technical report does not report on spatial spread, stem numbers, etc., but focuses instead on two assessments of colonization by herbivores. The first assessment involves colonization by the introduced plum mealy aphid, *Hyalopterus prunii*; the second assessment involves colonization by two introduced stem-mining flies, *Lipara similis* and *L. rufitarsis*. Based on field observations, it appears that introduced and native individuals will exhibit distinctly different growth strategies, levels of herbivore colonization, and expansion rates. To the authors' knowledge, this study documents the first clonal wetland plant common garden of its scale, as well as the largest consolidated collection of *P. australis* populations.

Figure 4. Experiment to study clonal expansion rates of native and introduced *P. australis*. Panel on left shows common garden design using linear "trenches" in July 2008 during construction. Right panel shows growth after 1 year (November 2009).

Table 5. Population origin, status (native or introduced haplotype), and haplotype (where known) used in the long-term growth and competition experiment.

| Population | Type | Haplotype |
|---|---|---|
| Antioch CA | Nat | PQ |
| Novato CA | Intro | M |
| Bergen Swamp NY | Nat | - |
| Rochester Hwy NY | Intro | - |
| Clark Co SD | Nat | - |
| SD Pop 1 | Intro | - |
| Darr Bridge NE | Nat | - |
| Darr Bridge NE | Intro | - |
| Deer Creek NY | Nat | - |
| Deer Creek NY | Intro | - |
| Dieppe NB | Nat | E |
| Mockton NB | Intro | M |
| Marenisco MI | Nat | E |
| Escanaba MI | Intro | M |
| Libby River ME | Nat | - |
| Libby River ME | Intro | - |
| Machinaw City MI | Nat | - |
| Long Lake MI | Intro | M |
| Montezuma NY | Nat | E |
| Montezuma NY | Intro | M |
| Pipewort IN | Nat | AB |
| Mile 59/60 IN | Intro | M |

| Population | Type | Haplotype |
|---|---|---|
| Seminary Fen MN | Nat | S |
| MN | Intro | - |
| Sun Lake WA | Nat | D |
| Moses Lake WA | Intro | M |
| TNC Choptanc MD | Nat | AD |
| TNC Choptanc MD | Intro | M |

Aphid populations were sampled on 2 and 3 August 2011 by randomly selecting two leaves from each plant to score aphid and predator abundance. Stems of average height were chosen within each plant, and the fourth leaf below the stem apex was then collected from each stem. To account for differences in aphid densities and clonal expansion, one leaf each from both the edge and center of each clone was sampled. Leaf samples with aphids attached were immediately frozen for future counting and analyses. During the next weeks and months, a dissecting microscope was used to identify and count every herbivore (winged and non-winged aphids) and predator/parasitoid (gall midges, syrphid larvae, two different mummy species) present on each leaf.

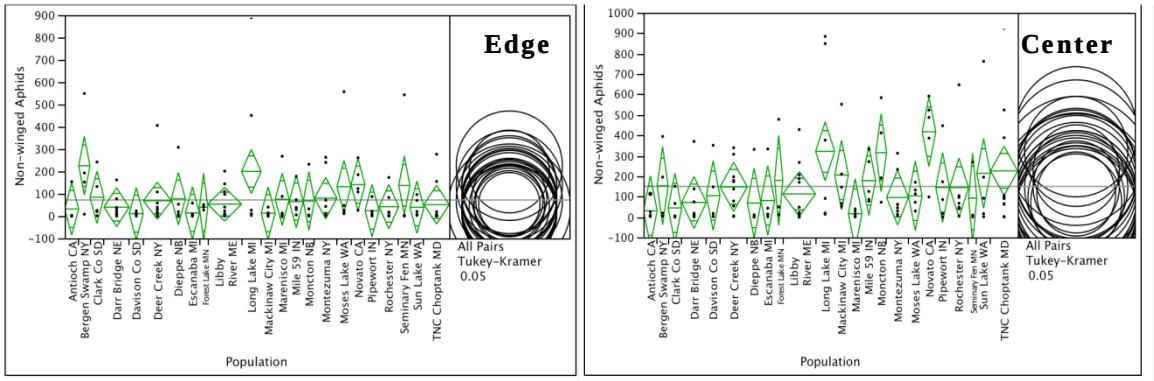
After counting enormous numbers of aphids and conducting exploratory statistical analyses (largely completed, but not entirely), findings are as follows:

1. The age of the attacked tissue matters, as aphids are significantly more abundant in the center of clones than at the edges (Figure 5). The location within a clone is the single largest explanatory variable.
2. Origin (native or introduced clone) and clonal identity (population and collection location) are not significant factors; i.e., aphid colonization proceeds independent of origin. There are also no significant differences in aphid abundance based on clonal identity.

Enormous variations were still encountered between aphid colonization, anecdotal reports collected in the field, and published reports from greenhouse locations (Lambert and Casagrande 2007). These variations suggest differences in attack rates based on plant origin. However, study results are the outcome of a number of ecological interactions and not exclusively a result of plant-aphid interaction. Large numbers of coccinellid and syrphid larvae were encountered, along with at least two different parasitoid species making different mummies, and other

predators. The susceptibility of *P. australis* and its potential impact on growth are not reported here, but the data collected do not indicate a difference in aphid numbers based on origin or population.

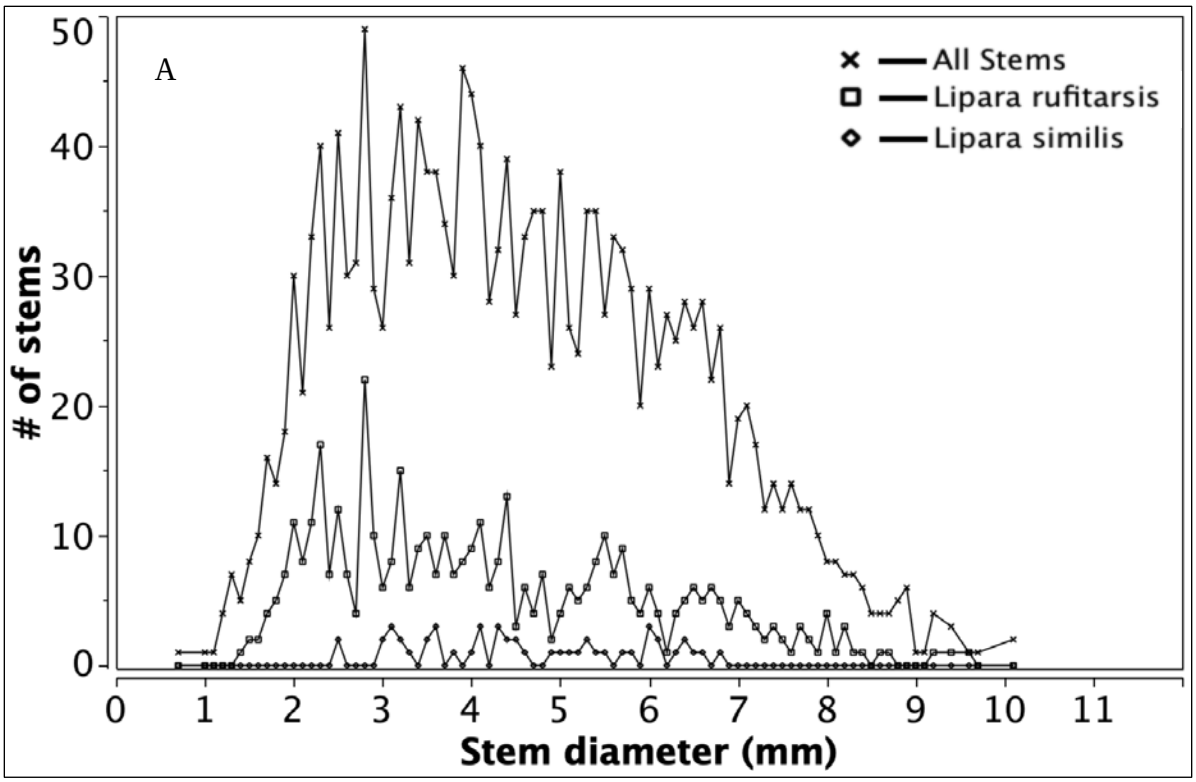
Figure 5. Aphid (*Hyalopterus pruni*) abundance on various *P. australis* genotypes at the edge or center of clones grown in a common garden. Data shown are means± SE and range of five clones for each population collected in July 2009.

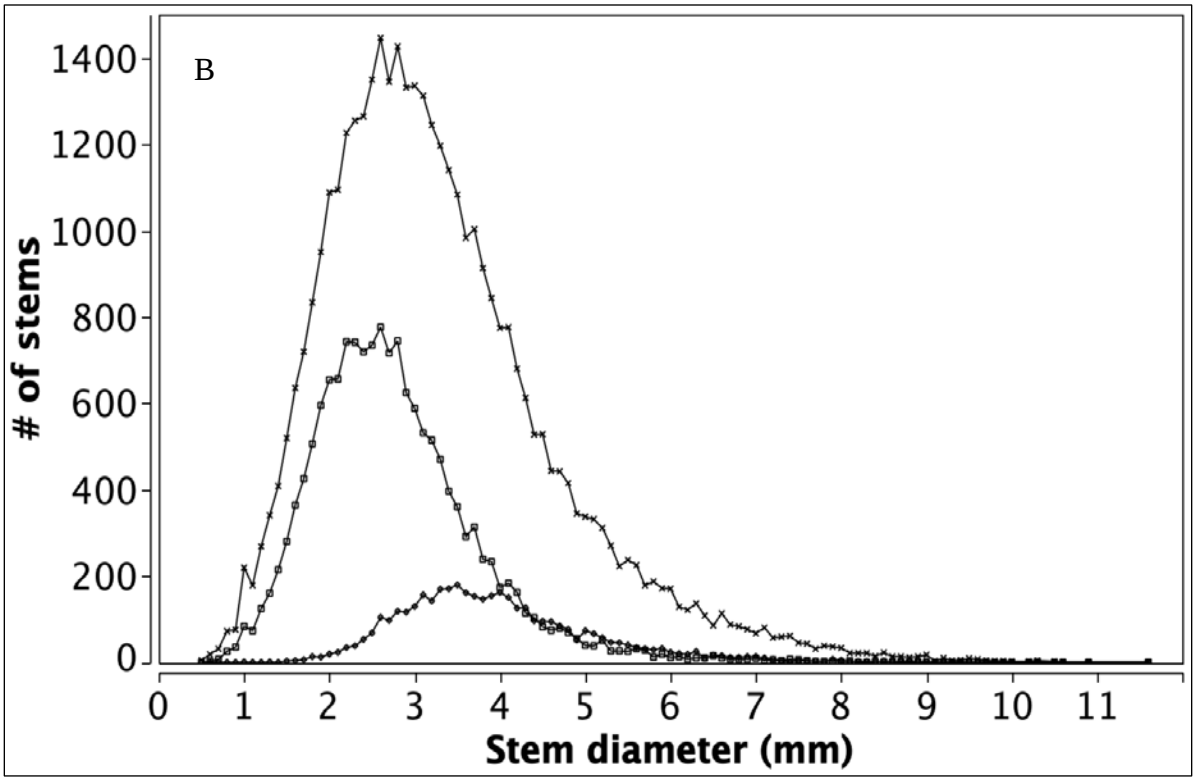



All stems of the 2010 growing season were harvested (in 1-m-long sections) before snowfall and stored in an outdoor sheltered location. Over the winter months, herbivore attack rates of more than 40,000 stems were measured and more than 4,000 individual stems were dissected. This report focuses on a few select results obtained for two stem-mining flies, *Lipara rufitarsis* and *L. similis*. Both species were introduced from Europe (Tewksbury et al. 2002) and are now widely distributed in the Great Lakes Region as far west as Michigan. These species can reach high attack rates and at one field site in central New York, they appeared to prefer native stems over introduced stems (Park and Blossey 2008).

These results confirm results from Europe, which indicated that different *Lipara* species prefer stems with different stem diameters (Figure 6) (Abraham and Carstensen 1982, Reader 2003). For *L. rufitarsis*, females prefer to oviposit on stems of smaller diameters and they have a more narrow range. *L. similis* prefers stems with a larger diameter and shows a larger range of acceptable diameters. In the common garden, introduced *Phragmites* clones expanded rapidly, reaching extremely high densities of several hundred per 0.5 $m^2$, which allowed a much larger sample size for introduced stems. A preliminary examination (further statistical details will be provided in a subsequent report) appears to suggest that the diameter preferences are similar for native and introduced stems (Figure 6).

Figure 6. Distribution of *P. australis* stems as a function of stem diameter (mm) for native stems (Figure 6A; N = 1,875), introduced stems (Figure 6B; N = 39,822), and stems that were attacked by *Lipara rufitarsis* or *Lipara similis*. Data are for the 2010 growing season, with all plants grown under standardized conditions in experimental trenches. Please note different y-axes.



Comparing the impact of *Lipara* spp. attack on stem growth showed a clear reduction in stem height for both native and introduced *P. australis* (Figure 7). This attack is nearly identical across a range of stem diameters. The attack did not appear to suppress growth by native or introduced clones differently. Thus, attack by *L. similis* and *L. rufitarsis* reduced plant height and eliminated seed output on attacked stems. Until recently, seed production in *P. australis* was considered of minor importance; however, recent work suggests that much fertile seed can be produced that will make a significant contribution to the regional and long-distance spread of *P. australis*. Thus, these two stem-mining flies could have some importance in the biological control of *P. australis* if their seed-suppressing effects have an impact on plant demography.

When the attack rates of *L. similis* and *L. rufitarsis* were compared as a function of stem density, a clear difference between the two species was evident. While attack rates for *L. rufitarsis* increased with increasing stem densities, *L. similis* attack rates were constant across the range of stem densities encountered in sampling (Figure 8). Thus, as a biocontrol agent, *L. rufitarsis* would appear to be a much more potent agent, responding positively to increasing host plant density and reaching higher attack rates across the entire spectrum of stem diameters (Figure 8).

The research summarized herein is still under development; dissections and data entry were only recently completed. The data collected will be further evaluated and analyzed, and a subsequent report will provide more detailed analyses.

Figure 7. *P. australis* stem height of native stems (Figure 7A, N = 1,875 stems) and introduced stems (Figure 7B, N = 39,822 stems), attacked (dashed lines) by *Lipara* spp. and unattacked (solid line), as a function of stem diameter (mm). Data were collected from plants grown in a common garden under identical conditions.

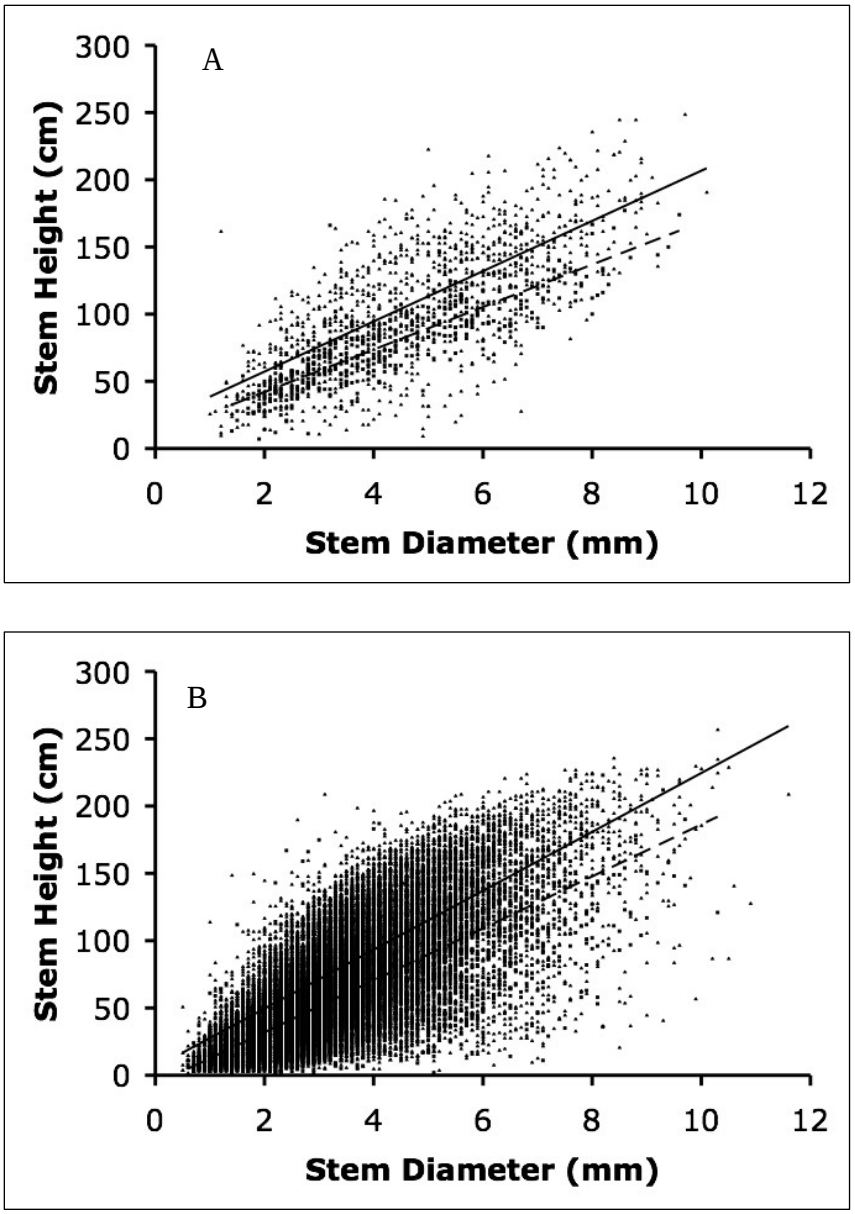
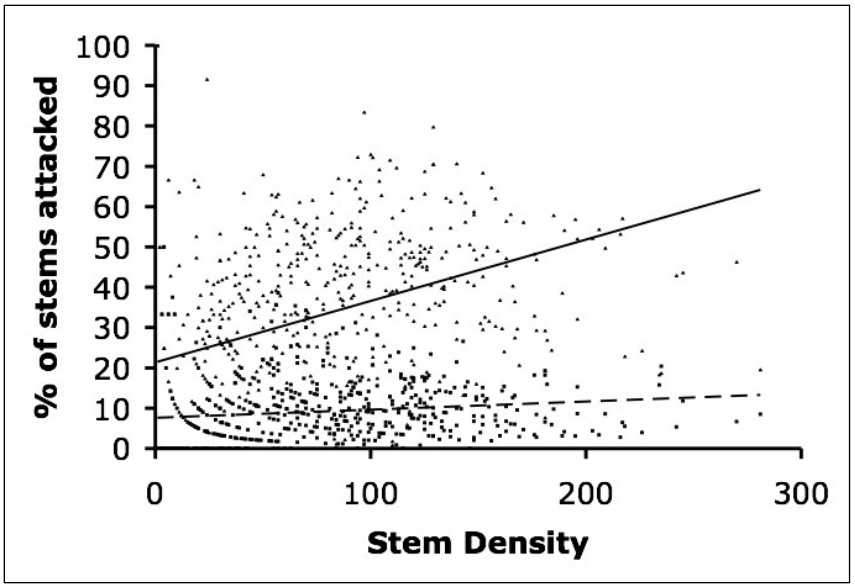


Figure 8. Attack rates (%) by *Lipara similis* (dashed line) and by *Lipara rufitarsis* (solid line) of *P. australis* stems (N = 41,697 stems) as a function of stem densities in trench sections (1 m long x 0.5 m wide, N= 75 trenches).



# 4 Objective 3: Develop Laboratory/ Greenhouse Mass-Rearing Procedures

In weed biocontrol programs, control agents are typically in very short supply during initial host specificity testing or even once field release permits are obtained. Traditionally, releases are made into field sites intended to serve as future "nursery sites;" i.e., sites where control agents were collected after populations had built sufficiently. This approach is problematic for various reasons, including the potentially long waiting period for control agents to build high populations, the danger of making poor choices for field release sites, and the loss of "momentum" in a control program. The purple loosestrife control program has been an interesting counter-example. The very first introductions were spread across the continent, included many different collaborators in different climate zones, and involved experiments to learn about the best release procedures (Hight et al. 1995). Within a few years of the initial release, mass production procedures had been developed. Some of these procedures were so simple that high school students were able to participate in the process (Blossey and Hunt 1999, Blossey et al. 2000) and distribute written and video guidelines on mass production.

Using the example of the purple loosestrife program, various mass-production techniques for the *P. australis* insects were evaluated during host specificity screening. The feeding mode of the stem-boring noctuids does not appear to be suitable for an easy transition to volunteer rearing, but the development of year-round rearings on artificial diet could greatly accelerate population build-up and facilitate distribution. Experimentation with such procedures has begun; existing knowledge will be summarized when trials during the ongoing field season are completed and evaluated. These will include both indoor and outdoor rearing attempts and partial development of a semi-artificial diet. Rearing on cut shoots was attempted in Europe, while artificial diet developments are being explored in Rhode Island. Initial results for artificial diet are not encouraging, as larvae struggle to reach later instars on various diet formulations. Research may need to focus more on rearing of sufficient quantities of eggs using the field-cage system and cut stem technique developed by P. Häfliger at CABI. Results of this research will be summarized in a subsequent report.

The possibilities of assessing host-specificity rearing procedures and their linkages to mass production assessments will also be discussed. One important point in completing host-specificity rearing is to allow larvae to reach the final instar and pupation. This would greatly reduce reliance on egg shipments from Europe. While the diameter and acceptance of *P. australis* stems has increased significantly, difficulties with final instars and their need for very large stem diameters continue. The ability to grow stems of very large diameters using fertilizer and flooded plants will continue to be monitored; however, plants often need 2 years to reach such size. A single cutting event may also set plants back a year; thus, there is a constant need for fresh and large materials. This need is taxing the capabilities of the research, particularly in quarantine, despite the part-time hire of a professional plant propagator for the project. Again, experiments are ongoing at this time and more data will be provided in a subsequent report.

# 5 Objective 4: Assist In Selecting Pre-Release Sites for Long-Term Monitoring

In collaboration with land managers, a number of *Phragmites* sites have been selected as long-term study sites in Rhode Island and New York (N=14 paired). These sites currently serve as monitoring sites to assess *Phragmites* expansion rates (native and introduced) until control agents are available; after that point in time, they will serve as release sites. Paired sites have similar habitat types and are located in the same geographic region. One of the two sites will serve as a future insect release site, while the other functions as a control (at least initially until insects disperse widely on their own). Sites are located sufficiently distant from each other (5-0 km) to prevent immediate dispersal of control agents among sites. Ideally, the research will cover a range from dry to flooded and from freshwater conditions to tidal full salinity salt marshes where *Phragmites* is able to grow. Where possible, additional control sites will be located where native *P. australis americanus* occurs to assess the status of this species over time. Once control agents have reached higher abundances, release sites can also serve as collection sites for redistribution of control agents. However, separate release sites are also anticipated as control agents become more widely available.

From August–September 2009, 15-20 permanent 1-$m^2$ quadrats were established along multiple transects through each *P. australis* clone that spans the "invasion front" of native or non-native clones. This allows the expansion rates of each clone (if any) to be assessed and the rates of spread among native and introduced clones to be compared. In addition, the presence and cover of all plant species within each quadrat were recorded. Field sites in New York were surveyed extensively for their plant communities in 2009 and 2010 (data summaries will be provided in forthcoming reports) and for their insect communities in *Phragmites* in Rhode Island. Plant community composition across the invasion gradient will be analyzed to determine whether patterns emerge from the multi-year dataset.

These research sites will also function as field sites for collaborating with personnel from the Cornell University Department of Plant Pathology, who will be studying how microbial diversity changes with *P. australis*

invasion at some of the sites (this work will be funded through a separate grant). This will allow for testing of assumptions and hypotheses about regulation of plant communities and invasions by microbial organisms. It may also offer the opportunity to identify additional biocontrol agents if certain microbial organisms demonstrate pathogenicity against seedlings or established plants.

# 6 New Objective 5: Assessing the Extent of Hybridization Between Native and Introduced Genotypes

Hybridization between native and introduced *P. australis* genotypes has been discussed as a possibility by researchers for a long time. Recently, the possibility of such events has received increased attention with the report that such hybrids can be created/forced in the laboratory (Meyerson et al. 2008). However, until recently, the existence of hybrids in the field has not been reported, despite some significant sampling efforts. There is now reliable genetic evidence of a hybrid occurrence in the field at the Montezuma National Wildlife Refuge (Saltonstall et al. 2014). The location of this hybrid is in the vicinity of two "parental" clones that grow close to the Visitor Center. Further searches in the larger Montezuma wetlands complex have revealed the existence of further "morphologically suspicious" individuals but these do not appear to be hybrids. The existence of hybrids not only makes development of biological control more difficult; it also complicates the management of the species using "traditional" means. The extent of possible hybridization was assessed regionally by visiting known locations in the Great Lakes region where both native and introduced clones exist or have been reported. Collaborators and contacts in the region helped in submitting samples for genetic analyses, but the existence of a field hybrid appeared to be restricted to a singly hybridization event (Saltonstall et al. 2014).

# 7 Outlook

Substantial progress has occurred in the work program discussed herein. Teams of students have dissected and catalogued more than 40,000 *P. australis* stems and this information is being used in graphical and statistical analyses. This work will continue and further updates will be publicized as they occur. A number of publications are anticipated as a result of this research, including reports on work being conducted in the area of host specificity.

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**14. ABSTRACT**

Introduced *Phragmites australis* is rapidly spreading in North America, threatening wetland plant communities and endemic native genotypes (*Phragmites australis americanus*). Lack of successful long-term control resulted in initiation of biological control research. In the past, the program targeting introduced *Phragmites* has focused on several promising potential biological control agents with large impacts on *P. australis*. The purpose of this report is to: (1) identify potential agents for in-depth study; (2) outline and report initial testing procedures and results of host-specificity studies of identified agents; (3) assess possibilities to develop laboratory/greenhouse mass-rearing procedures; (4) outline approaches for long-term monitoring at pre-release sites; and (5) assess the extent of hybridization between native and introduced genotypes. All selected insect species are stem miners that overwinter as eggs, with larvae feeding in spring and early summer. Host specificity testing is being conducted in a Rhode Island quarantine facility and at the Center for Agricultural Bioscience International (CABI) in Switzerland. In addition, investigations continue on the impact of *Phragmites* populations on native fauna and flora as well as the economic and ecological effects of *Phragmites* invasion. Hybridization between native and introduced genotypes appears to be restricted to a single hybridization event in central New York State.

**15. SUBJECT TERMS**

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